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COMPLETE SPECIFICATION

Title of Invention:
Process for producing composition containing bovine insulin-like growth factor 1

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SPECIFICATION

A METHOD OF PREPARING A COMPOSITION CONTAINING BOVINE INSULIN-LIKE GROWTH FACTOR-1

Technical Field

The present invention relates to a method of preparing a composition containing insulin-like growth factor-1. The composition containing insulin-like growth factor-1 has a bone reinforcement effect, and is useful for preventing and treating osteoporosis.

Background Art

In recent years, patients suffered from a variety of bone diseases such as osteoporosis, fracture and lumbago have increased with the increase of the aged people. It is deemed to be resulted from the lack of the intake of calcium, the lowering of digestive ability of the calcium, hormone unbalance postmenopause and the like. In order to prevent the various bone diseases such as osteoporosis and fracture with the increase of the aged people, it is deemed to be effective to increase bonemass as much as possible so as to improve the peak bonemass. The improvement of the peak bonemass is no other than the reinforcement of bones.

Under such circumstances, calcium salts such as calcium carbonate, calcium lactate and calcium phosphate, and natural calcium agents such as bovine bone powder, egg shell and fish bone powder are used in order to supplement calcium. However, some of the calcium agents form insoluble salts in the digestive tract, and such calcium agents are not absorbed

in the bodies sufficiently. In addition, as medicals for the treatment of osteoporosis and for bone reinforcement, vitamin D_3 and calcitonin formulations are used. However, when these medicals are used, side effects such as ear noises, headache and anorexia will be produced. Therefore, it is desired the development of a food which can be administered orally for a long period and which can be expected the treating effects of osteoporosis in view of the characters of the diseases of osteoporosis.

On the other hand, It has been known that the insulin-like growth factor-1 (it will be abbreviated as "IGF-1" hereinafter) is a polypeptide having a molecular weight of about 7.800 and belonging to somatomedin class, and that it plays an important role in the bone metabolism, e.g., it activates the osteoblast and increases the bonemass and then reinforces the bones. Further, it was found recently that a receptor of the IGF-1 exists in digestive tract, and it was suggested that the orally administered IGF-1 acts via the receptor in the digestive tract (Hormones and Clinics, 39, pp.31-37, 1991). As to the IGF-1, its existence in the human milk was confirmed (J.Clin.Endocrinol.Metab., 58, pp.955-959, 1984), and in addition to it, its existence in blood serum and all organs such as liver was confirmed (Proc.Natl.Acad.Sci.USA, 81, pp.935-939, 1984).

Further, as a method of preparing the IGF-1, a method of isolating it from blood serum (J.Biol. Chem., 261, pp.569-575,1986), and a method of producing it by genetic recombination (Japanese Patent Application (OPI) (the term

"OPI" as used herein means an unexamined published patent application) No.63-269984) and the like have been known. However, if it is considered that the IGF-1 is added to foods in order to prevent and treat osteoporosis, the IGF-1 isolated from serum or produced by genetic recombination has problems from the view points of cost and safety.

On the other hand, it has been confirmed that the IGF-1 exists in bovine milk, and it was reported that the chemical structure of the bovine IGF-1 is just the same as the human IGF-1 (Biochem.J., 251, pp.95-103, 1988). It suggests that the bovine IGF-1 has the same activities as the human insulin-like growth factor-1, and as far as the bovine IGF-1 exits in bovine milk, there is no problem at all in safety even if it is added to foods.

As a method of preparing the bovine IGF-1 from bovine milk, the method comprising treating the bovine colostrum by a method combining cation exchange chromatography, gel filtration, reverse phase HPLC and the like after acid extracting it, has been known (Biochem.J., 233, pp.207-213, 1986). However, the process of the method are practically complicated, and in particular, most of the milk proteins are acid precipitated by the acid extraction of the bovine colostrum, and the acid precipitated milk proteins can not be utilized effectively. Thus the method can not deemed to be an industrially preferable method. Further, a method of isolating a peptide, which is lacking in five residues of the N-terminal of the bovine IGF-1, from the bovine milk has been known (Japanese Patent Application (OPI) No.63-501567).

However, the method also comprises acid precipitation and cation exchange chromatography and the like, and thus the method is not an industrially preferable method, as the above method.

Previously, the present inventors proposed a method of preparing the composition containing IGF-1 by thermal treatment of bovine milk or a raw material derived from the bovine milk (Japanese Patent Application (OPI) No. S-97273). However, the IGF-1 content of the composition containing the IGF-1 was about 10 to 25μg/g, so that a method of preparing a composition containing a higher amount of the IGF-1 have been desired. In addition, even in the method, there was a problem that the milk proteins precipitated by heating could not be utilized efficiently.

The present inventors, in view of the above-mentioned problems, have researched earnestly on a method of separating the bovine IGF-1 from the bovine milk or the raw material derived from the bovine milk, and found that a composition containing a high amount of the bovine IGF-1 may be obtained by using a cation exchanger, and completed the present invention. Therefore, an object of the present invention is to provide a method of separating a composition containing a high amount of bovine IGF-1 which has the same chemical structure as the human IGF-1 and is useful for bone reinforcement and for preventing and treating osteoporosis, efficiently from bovine milk.

Disclosure of Invention

In the present invention, when a composition

containing a high amount of bovine IGF-1 is prepared, bovine milk or a raw material derived from the bovine milk is contacted with a cation exchanger to adhere the bovine IGF-1 fraction, then the fraction is eluted with an appropriate eluting solution, and the bovine IGF-1 is recovered, wherein the salt concentration of the eluting solution used in the elution is in the range from 0.1M to 0.3M, and the pH of the eluting solution is in the range from 5.6 to less than 8.

The bovine milk or the raw material derived from the bovine milk used in the present invention is, for example, skim milk, cheese whey, acid whey or colostrum. In addition, the reconstituted whey protein concentrates (WPC), whey protein isolates (WPI), whole powder milk, skim powder milk, whey powder and the like may be used. Further, these materials are preferably preheated before contacting them with cation exchanger. That is, by heating the materials so as to denature the major milk proteins such as casein, α-lactalbumin, β-lactoglobulin existing in the milk, the amount of impurities adhered to the cation exchanger may be decreased. As the results, the content of the bovine IGF-1 contained in the composition is improved. Even if the heating treatment is not carried out, the bovine IGF-1 can be recovered sufficiently, but high amounts of casein and whey proteins are included so that the IGF-1 content in the composition is decreased. When the heating treatment is carried out, the heating temperature may be determined according to the following formula:

$$T \geq -5\,(pH) + 100$$

(wherein, T shows a centigrade, and pH is in the range $2 \leq pH \leq 7$).

The pH of the raw material contacting with the cation exchanger is not limited particularly, but if the pH is

too low, most of the included milk proteins adhere to the cation exchanger, and as the results, the bovine IGF-1 content in the composition is decreased. On the contrary, if the pH is too high, the amount of the bovine IGF-1 adhered to the cation exchanger is decreased, and thus it is not preferable. Therefore, it is preferable to carry out the cation exchange treatment at the neutral pH range similar to the normal bovine milk.

In addition, the salt concentration of the raw material contacting with the cation exchanger is not limited particularly, but if the salt concentration of the raw materials is too high, the adhesion to the cation exchanger is lowered as the conventional ion exchange treatment, and the recovery rate of the bovine IGF-1 from the raw materials is lowered. Accordingly, it is preferable to adjust the salt concentration to the same as or less than the salt concentration of the normal bovine milk.

Further, when the bovine milk or the raw material derived from the bovine milk is contacted with the cation exchanger, it is preferable to treat it previously with a clarifier so as to remove fine precipitates contained in the raw materials.

In the present invention, the method of contacting the bovine milk or the raw material derived from the bovine milk with the cation exchanger is not limited particularly, and the cation exchange may be carried out according to a conventional methods such as a method using a filling layer type column, a method using a rotating column, or a batch

method. In addition, the contacting time of the bovine milk or the raw material derived from the bovine milk with the cation exchanger is not limited particularly, and the longer contacting time is more preferable. However, if the contacting time is too long, the raw material is deteriorated, so that the contacting time is desirably in the range from 10 minutes to 24 hours. Further, the temperature of the raw material contacted to the cation exchanger is not limited particularly, but the range from 4°C to 40°C is desirable. If the temperature is 40°C or more, the raw material will be deteriorated conspicuously.

In the present invention, the ratio of the cation exchanger to the raw material is the cation exchanger/the raw material=1/10 (w/w) to 1/3,000(w/w), preferably the cation exchanger/the raw material=1/16 (w/w) to 1/1,000(w/w). The value of the cation exchanger/the raw material is higher than 1/10(w/w), the cost of the cation exchanger will increase relatively. If the value of the cation exchanger/the raw material is less than 1/3,000, the recovery rate of the bovine IGF-1 will decrease extremely.

As the cation exchanger which may be used in the present invention, CM-Cellurofine, CM-Cellurose, Microplep CM Strong Cation Exchange Support, CM-Sepharose, CM-Sephadex and C-Spherosil having a carboxy methyl group as an exchange group, Sulfonated Chitopearl, SP-Toyo Pearl, S-Sepharose, SP-Sephadex, Indion S3, S-Spherosil and Microplep S Strong Cation Exchange Support having a sulfonic group as an exchange group, may be exemplified. However, in order to obtain a composition

containing a higher amount of the bovine IGF-1. it is desirable to use strong acid cation exchanger having a sulfonic group as an exchange group.

In the present invention, after contacting the bovine milk or the raw material derived from the bovine milk with the cation exchanger to adhere the bovine IGF-1 to the cation exchanger, the cation exchanger is washed with a solution having a salt concentration of less than 0.1M salt concentration or with deionized water. The washing treatment is preferably carried out, since part of the included milk protein can be removed from the cation exchanger if the washing is carried out, and as the results, the bovine IGF-1 content in the composition may be increased. After that, the bovine IGF-1 is eluted from the cation exchanger. As the eluting method, the eluting may be carried out according to the usually adopted eluting method. However, the salt concentration of the eluting solution used in the eluting should be in the range from 0.1M to 0.3M. If the salt concentration of the eluting solution is less than 0.1M, the elution of the bovine IGF-1 from the cation exchanger can not be made sufficiently. If the salt concentration of the eluting solution exceeds 0.3M, the proteins other than the bovine IGF-1 adhered to the cation exchanger will be eluted at the same time, so that the content of the bovine IGF-1 in the composition is decreased.

As for the pH of the eluting solution, it was found, by our experiments, that the range from 5 to less than 8 is preferable. Therefore, the eluting solution may be a solution

of a neutral salt such as sodium chloride, potassium chloride and ammonium acetate, dissolved in a conventional buffer solution such as Tris-hydrochloric buffer solution, phosphoric buffer solution, carbonic buffer solution. In addition, as the eluting solution, a neutral salt solution having a salt concentration in the range from 0.1M to 0.3M and having no buffer ability may be used, and such eluting solution is preferable in view of the operational improvement and the cost reduction.

Then, thus obtained eluate containing the bovine IGF-1 fraction may be desalted and concentrated by a conventional method such as a method using ion exchange resin, reverse osmosis membrane, ultrafiltration membrane, dialysis membrane, electrodialysis membrane or gel filtration carrier, or by a method combining these methods. However, as the desalting and concentrating method, a method combining the dialysis and the diafiltration is preferable since the concentrating and desalting may be carried out at the same time. The ultrafiltration membrane which may be used in such case may be any ultrafiltration membrane, provided that the fractional molecular weight thereof is 10kDa or less. The composition concentrate containing bovine IGF-1 may be used as it is, but depending on the necessities, dry powder of the composition containing bovine IGF-1 may be obtained by a method such as spray drying or freeze drying. Further, a step of a conventional thermal sterilization may be added, since the bovine IGF-1 has a relatively thermal stable characteristics.

Measuring the bovine IGF-1 content in the bovine IGF-1 contained in the composition obtained by the method of the present invention, by a immunological measuring method using an anti-IGF-1 antibody, the recovery rate of the IGF-1 from the raw material was 40% in average. On the other hand, the literature discloses that the recovery rate of the bovine IGF-1 recovered by a method combining acid extraction and cation exchange chromatography from colostrum is 25% (Biochem. J., 233, pp.207-213, 1986). Thus the recovery rate by the method of the invention is greater than the method.

In addition, the present invention is more useful than the method combining the acid extraction and the cation exchange chromatography, since the milk components which is not adhered to the cation exchanger may be utilized again and the processes are not complicated by the present invention.

In the composition containing bovine IGF-1, components such as casein or whey proteins are contained, and in particular, the proteins such as lactoferrin, lactoperoxidase, secretory components have physiological activities. However, since these proteins have no effect on the physiological activities of the bovine IGF-1, there is no problem even if these proteins are contained, but if there is inconvenient, these proteins may be deactivated by a treatment such as heating. In addition, as for the lactoperoxidase, it may be separated by a method such as rechlomatography or converted into acid to deactivate.

The composition containing bovine IGF-1 obtained by the present invention has a bone reinforcement effect, so that

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it may be added to beverages, foods, medicals, animal feeds so as to provide preventing and treatment effects for osteoporosis. In addition, these effects may be increased by adding the calcium agents having good absorptive properties, such as calcium chloride, calcium carbonate, calcium lactate, egg shell or calcium derived from milk, to the beverages, foods, medicals and animal feeds containing the composition containing bovine IGF-1. As for the composition containing bovine IGF-1, an acute toxicity was not found as the result of an animal experiments using rats.

Brief description of Drawings

Fig.1 shows the osteoblast growth promoting effects of the composition containing bovine IGF-1 obtained in the following Examples 1 to 9.

Fig.2 shows the bone reinforcement effect of the composition containing bovine IGF-1 obtained in the following Example 5.

Examples

The present invention will be explained in more detail by way of Examples hereinafter.

Example 1

50L of cheese whey (pH6.0) which had been heated to sterilize at 150°C for 5 sec. was run through a column, filled with 50g of sulfonated Chitopearl (manufactured by Fuji Boseki Co., Ltd.) which had been washed sufficiently with deionized water, at a running rate of 25ml/min. After running through, the sulfonated Chitopearl was washed with deionized water sufficiently, then the adhered bovine IGF-1 was eluted with

0.02M carbonic buffer solution (pH7.0) containing 0.28M sodium chloride. Then, after desalting and concentrating the eluate with an ultrafiltration membrane having a fractional molecular weight of 10kDa, it was freeze-dried to obtain 450mg of powdered bovine IGF-1 containing composition. Measuring the content of the bovine IGF-1 contained in the composition containing bovine IGF-1 with radioimmunoassay (RIA), it was found to be 160μg/g.

Example 2

1,000L of non-sterilized skim milk (pH6.5) was run through a column, filled with 1kg of SP ToYo Pearl (manufactured by Toso Co., Ltd.) which had been washed sufficiently with deionized water, at a running rate of 30ml/min. After running through, the SP ToYo Pearl was washed sufficiently with deionized water, then the adhered bovine IGF-1 was eluted with 0.05M carbonic buffer solution (pH7.5) containing 0.15M sodium chloride. Then, after desalting and concentrating the eluate by carrying out ultrafiltration and diafiltration with a membrane having a fractional molecular weight of 8kDa, it was freeze-dried to obtain 50g of powdered composition containing IGF-1. Measuring the content of the bovine IGF-1 contained in the composition containing bovine IGF-1 with radioimmunoassay (RIA), it was found to be 65μg/g.

Example 3

Whey protein concentrates (WPC) was dissolved in distilled water sufficiently so as to have a concentration of 10 weight%, and 40L of whey protein solution (pH6.8) was prepared. The whey protein solution was run through a column,

filled with 400g of CM-Cellurofine (manufactured by Seikagaku Kogyo Co., Ltd.) which had been washed sufficiently with deionized water, at a running rate of 20ml/min. After running through, the CM-Cellurofine was washed sufficiently with 0.03M phosphate buffer solution (pH7.4) containing 0.02M sodium chloride, then the adhered bovine IGF-1 was eluted with 0.10M citric buffer solution (pH6.2) containing 0.20M sodium chloride. Then, after desalting and concentrating the eluate by electrodialysis (ED) method, it was freeze-dried to obtain 1.3g of powdered composition containing bovine IGF-1. Measuring the content of the bovine IGF-1 contained in the composition containing bovine IGF-1 with radioimmunoassay (RIA), it was found to be 35μg/g.

Example 4

Whey protein isolates (WPI) was dissolved in distilled water so as to have a concentration of 10 weight%, and 80L of whey protein solution (pH6.5) was prepared. The whey protein solution was run through a column, filled with 800g of SP-Sepharose (manufactured by Pharmacia Co., Ltd.) which had been washed sufficiently with deionized water, at a running rate of 24ml/min. After running through, the SP-Sepharose was washed sufficiently with 0.05M carbonic buffer solution (pH7.6) containing 0.01M sodium chloride, then the adhered bovine IGF-1 was eluted with 0.20M citric buffer solution (pH5.7) containing 0.10M sodium chloride. Then, after desalting the eluate by ion exchange chromatography, it was freeze-dried to obtain 29.6g of powdered composition containing IGF-1. Measuring the content of the bovine IGF-1

contained in the composition containing bovine IGF-1 with radioimmunoassay (RIA), it was found to be 51.2μg/g.

Example 5

After 3t of acid casein whey, which had been heated to sterilize at 121°C for 30 sec, was adjusted to pH6.0 with sodium bicarbonate, it was run through a column, filled with 30kg of SP-Sephadex (manufactured by Pharmacia Co., Ltd.), which had been washed sufficiently with deionized water, at a running rate of 10L/min. After running through, the SP-Sephadex was washed sufficiently with deionized water, then the adhered bovine IGF-1 was eluted with 0.05M carbonic buffer solution (pH7.0) containing 0.25M sodium chloride. Then, after desalting and concentrating the eluate with reverse osmosis (RO) membrane, it was freeze-dried to obtain 176g of powdered composition containing bovine IGF-1. Measuring the content of the bovine IGF-1 contained in the composition containing bovine IGF-1 with radioimmunoassay (RIA), it was found to be 106μg/g.

Example 6

After whey protein concentrates (WPC) was dissolved in distilled water so as to have a concentration of 10 weight% and whey protein solution (pH6.8) was prepared, the solution was heated to 90°C for 10 min, and was centrifuged at 17,000G to obtain 10L of supernatant. The supernatant was run through a column, filled with 500g of Indion S3 (manufactured by Organo Co., Ltd.), which had been washed sufficiently with deionized water, at a running rate of 18ml/min. After running through, the Indion S3 was washed with 0.07M Tris-hydrochloric

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buffer solution (pH7.6) sufficiently, then the adhered bovine IGF-1 was eluted with 0.3M sodium chloride solution (pH7.3). Then, after desalting the eluate by gel filtration chromatography, it was freeze-dried to obtain 1.4g of powdered composition containing bovine IGF-1. Measuring the content of the bovine IGF-1 contained in the composition containing bovine IGF-1 with radioimmunoassay (RIA), it was found to be 42μg/g.

Example 7

2L of colostrum (pH6.8), which had been heated to sterilize at 150°C for 5 sec, was run through a column, filled with 100g of S-Spherosil (manufactured by IBF Co., Ltd.) which had been washed sufficiently with deionized water, at a running rate of 20ml/min. After running through, the S-Spherosil was washed sufficiently with deionized water, then the adhered bovine IGF-1 was eluted with 0.3M sodium chloride solution (pH7.3). Then, after desalting and concentrating the eluate by carry out ultrafiltration and diafiltration with a membrane having a fractional molecular weight of 10kDa, it was freeze-dried to obtain 1.5g of powdered composition containing bovine IGF-1. Measuring the content of the bovine IGF-1 contained in the composition containing bovine IGF-1 with radioimmunoassay (RIA), it was found to be 184μg/g.

Example 8

100L of skim milk (pH6.5), which had been heated to sterilize at 121°C for 30 sec, was run through a column, filled with 0.5kg of Microlep S Strong Cation Exchange Support (manufactured by Biorad Co., Ltd.), which had been washed

sufficiently with deionized water, at a running rate of 35ml/min. After running through, the Microlep S Strong Cation Exchange Support was washed sufficiently with 0.05M carbonic buffer solution, then the adhered bovine IGF-1 was eluted with 0.10M Tris-hydrochloric buffer solution (pH7.4) containing 0.20M sodium chloride. Then, after desalting and concentrating the eluate with reverse osmosis (RO) membrane, it was freeze-dried to obtain 1.3g of powdered composition containing bovine IGF-1. Measuring the content of the bovine IGF-1 contained in the composition containing bovine IGF-1 with radioimmunoassay (RIA), it was found to be 114μg/g.

Example 9

20L of non-sterilized cheese whey (pH6.5) was run through a column, filled with 300g of C-Spherosil (manufactured by IBF Co., Ltd.), which had been washed sufficiently with deionized water, at a running rate of 25ml/min. After running through, the C-Spherosil was washed sufficiently with deionized water, and further washed sufficiently with 0.07M phosphoric buffer solution (pH7.2), then the adhered bovine IGF-1 was eluted with 0.05M citric buffer solution (pH6.5) containing 0.25M sodium chloride. Then, after desalting and concentrating the eluate with nanofiltration membrane, it was freeze-dried to obtain 890mg of powdered composition containing bovine IGF-1. Measuring the content of the bovine IGF-1 contained in the composition containing bovine IGF-1 with radioimmunoassay (RIA), it was found to be 43μg/g.

Test Example 1

The osteoblast growth promoting effects of the composition containing bovine IGF-1 obtained in Examples 1 to 9 were tested.

The cultured osteoblast-like strains (MC3T3-E1) were inoculated on a flat-bottomed cell culture plate having 96 holes, and they were cultured with α-MEM medium (manufactured by Flow Laboratories Co., Ltd.) containing 0.3 weight% of bovine serum for 18 hours. On the culture, 2μl of a solution of the composition containing bovine IGF-1 dissolved in 100μl of the medium in the concentration of 0.5 weight%, was added. After the culture, ^{3}H-labelled thymidine was added, and the radio activities of the ^{3}H-thymidine entrapped in the cells were measured after two hours in order to determine the growing activities of the osteoblast. The results will be shown in Fig.1. In Fig.1, the radio activity of the group having no composition containing bovine IGF-1 in the medium was made 100%, and the osteoblast growth promoting activities of the groups having the composition containing bovine IGF-1 were shown from the radio activities thereof. By the results, the osteoblast growth promoting activities of the groups having the compositions containing bovine IGF-1 obtained in Examples 1 to 9 showed 1.8 to 2.7 times as much as that of the group having no composition containing bovine IGF-1.

Test Example 2

The bone reinforcement activity of the composition containing bovine IGF-1 obtained in Example 5 was determined by an animal experiment.

As the experiment animals, 4 weeks old, SD strain

female rats were used. After prebreeding these rats for one week, the animals were ovariectomized, and further they were bred with the calcium deficient diet for five weeks, and were used for the experiments. In addition, siamese rats, which had undergone a false operation and had not undergone ovariectomy, were also used for the experiment. The osteoporosis-model rats were divided into three groups (n=7) comprising the control group (A group), the composition containing bovine IGF-1 administered group (B), and the composition containing bovine IGF-1 + calcium administered group (C), and each group were bred with the test diets shown in Table 1.

Table 1

	(A)Group	(B)Group	(C)Group
Sucrose	51.05(%)	51.46(%)	50.62(%)
Casein	20.0	18.0	18.0
Corn Starch	15.0	15.0	15.0
Cellulose	5.0	5.0	5.0
Corn Oil	5.0	5.0	5.0
Vitamin Mixture	1.0	1.0	1.0
Mineral Mixture	2.65	2.43	3.27
DL-Methionine	0.3	0.3	0.3
Composition containing Bovine IGF-1	-	1.81	1.81

1.81 weight% of the composition containing bovine IGF-1 was added in place of the nitrogen amount corresponding

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to 2 weight% of casein. The amounts of calcium, phosphor and magnesium in the diet were 300mg, 230mg and 50mg per 100g of the diet, respectively. Further, as for the (C) group, the amounts of calcium and phosphor were 520mg and 400mg per 100g of the diet, respectively.

After three weeks, femora of the both sides of the rats in each group were extirpated, and breaking force of femora was measured with a bone fracture properties measuring device. The results will be shown in Fig.2. By the results, the breaking force of femora of the composition containing bovine IGF-1 administered group (B) showed a statistically significant high value compared to the control group (A). Further, the composition containing bovine IGF-1 + calcium administered group (C) showed a statistically significant high value compared to the control group (B).

Reference Examples of the beverages and foods in which the composition containing bovine IGF-1 prepared by the method of the present invention are added, will be shown hereinafter.

Reference Example 1

In accordance with a conventional method, fruit juice beverage, which contains the composition containing bovine IGF-1 and has the composition shown in Table 2, was prepared.

Table 2

Mixed Isomerized sugar	15.0 (weight%)
Fruit Juice	10.0
Citric Acid	0.5

Composition containing bovine IGF-1	0.5
Flavor	0.1
Calcium	0.1
Water	73.5

Reference Example 2

In accordance with a conventional method, a calcium agent, which contains the composition containing bovine IGF-1 and has the composition shown in Table 3, was prepared.

Table 3

Water Containing Crystalline Glucose	73.5 (weight%)
Composition containing Bovine IGF-1	20.0
Calcium	5.0
Sugar Ester	1.0
Flavor	0.5

Industrial Applicability

With the method of the present invention, a bovine IGF-1 composition containing a high amount of bovine IGF-1 may be provided from bovine milk or a raw material derived from the bovine milk. Since the composition containing bovine IGF-1 has a bone reinforcement activity, the composition is useful for preventing or treating various bone and articulation diseases, in particular osteoporosis. In addition, by administering the bovine IGF-1 composition to human during their growing periods, the peak bonemass may be increased. Therefore, the composition containing bovine IGF-1 is useful

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as a raw material for beverages, foods, medicals and animal diets.

CLAIMS

1. A method of preparing a composition containing bovine insulin-like growth factor-1, comprising the steps of contacting bovine milk or a raw material derived from the bovine milk with a cation exchanger to adhere the bovine insulin-like growth factor-1, then eluting and recovering it, wherein the salt concentration of the eluting solution used in the elution is in the range from 0.1M to 0.3M, and the pH of the eluting solution is in the range from 5.6 to less than 8.

2. A method of preparing the composition as claimed in Claim 1, wherein the preheated bovine milk or raw material derived from the bovine milk is used.

3. A method of preparing the composition as claimed in any of Claims 1 or 2, wherein the cation exchanger is a strong acid cation exchanger having a sulfonic group as an exchange group.

4. A method of preparing the composition as claimed in any of Claims 1 to 3, further comprising the step of desalting and concentrating the eluate containing the bovine insulin-like growth factor-1 with an ultrafiltration membrane having fractional molecular weight of 10kDa or less.

END OF CLAIMS

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Fig.1